

In another aspect, the modification may provide means for improved attachment of capture molecules via a combination of one or more mechanisms, including covalent attachment, hydrophobic interactions, ionic interactions, hydrogen bonding, van der Waals interactions, or ligand binding mechanisms. In another aspect, the modification helps provide a water contact angle of between 60 and 75 degrees on the modified first surface. The modification of the first surface may be performed by using a number of different processes, such as plasma activation, chemical vapor deposition, liquid phase deposition, or surface polymerization of an activation chemistry. Many different chemicals may be used to modify the first surface of the planar waveguide. Examples of such chemicals include but are not limited to organosilane, alkoxysilane, chlorosilane, alkylsilane, epoxy silane, glycidoxysilane, aldehyde silane, aminosilane, or combination thereof. Specifically, glycidoxypropytrimethoxysilane or glycidoxypropytrimethoxysilane may be used as the modifying chemicals. Example polymer surface modifications include those based on polyethylene glycols, acrylate polymers, dextran, and combination thereof.

**[0056]** The term “capture molecule” is used here to describe any of a variety of molecules that could be attached to the first surface for performing a useful assay. The capture molecules may be a peptide, a polypeptide, a protein, an antibody, an antigen, an aptamer, a polysaccharide, a sugar molecule, a carbohydrate, a lipid, an oligonucleotide, a polynucleotide, a synthetic molecule, an inorganic molecule, an organic molecule, and combination thereof. The terms “polypeptide,” “peptide” and “protein” may be used interchangeably in this disclosure. The terms “oligonucleotide,” and “polynucleotide” may also be used interchangeably in this disclosure. For purpose of this disclosure, when referring to a polypeptide or a polynucleotide molecule, it is intended that either the full length molecule or a fragment of the full length molecule may be used. Moreover, any mutated forms of a polypeptide (antigen) or the DNA molecule encoding such a polypeptide are also within the scope of the disclosure, if such mutation or mutations do not reside within any epitope of the polypeptide (antigen), or if the mutation or mutations do not substantially decrease the binding affinity between the polypeptide (antigen) and a specific antibody against the polypeptide or a fragment thereof. Plural or singular forms of a noun may be used interchangeably unless otherwise specified in the disclosure. Capture molecules may also be in the form of a molecular mixture. For example, a cell lysate preparation containing a mixture of molecules may be attached to the first surface.

**[0057]** In one embodiment, the first surface of the planar waveguide may contain at least one reaction site (e.g., spot or stripe), wherein each of the reaction site may be formed by printing (i.e., spotting or depositing) a composition onto the first surface. In another embodiment, the first surface of the planar waveguide may have an array (also referred to as a “microarray”) of two or more reaction sites. In another aspect, the first surface may contain an array having four, five, six, seven, eight, nine, or ten reaction sites. In yet another aspect, the first surface may contain an array having between two and thirty reaction sites. In yet another aspect, the first surface may contain an array having between two and fifty reaction sites. In yet another aspect, the first surface may contain an array having between two and three hundred reaction sites. Each of reaction sites on the array may be formed by printing a composition onto the first surface. Each composition that is

printed onto each reaction site may contain one or more capture molecules. Typically, different reaction sites have different capture molecules. However, for the purpose of having replicate readings, multiple reaction sites may contain identical capture molecules.

**[0058]** For the purpose of this disclosure, the method and system described are based on assays that use fluorescence signal to quantify analyte(s) present in a sample. However, the embodiments described herein may be applicable to assays beyond fluorescence-based signal transduction. In addition, the method and system may also be compatible with luminescence, phosphorescence, and light scattering based signal transduction. In exemplary embodiments, excitable tags may be used as detection reagents in assay protocols. Exemplary tags include, but are not limited to, fluorescent organic dyes such as fluorescein, rhodamine, and commercial derivatives such as Alexa dyes (Life Technologies) and DyLight products; fluorescent proteins such as R-phycoerythrin and commercial analogs such as SureLight P3; luminescent lanthanide chelates; luminescent semiconductor nanoparticles (e.g., quantum dots); phosphorescent materials, and microparticles that incorporate these excitable tags. For the purpose of this disclosure, the term “fluorophore” is used generically to describe all of the excitable tags listed above.

**[0059]** Additionally, the first surface of the waveguide may include a pre-formed feature to serve as, for instance, a reaction site, such as an analyte detection site, a negative control site, a positive control site or a reference site. When two or more reaction sites are present, the array may be arranged such that the reaction sites are spread out on the first surface in rows and columns, with the distance between neighboring column and the distance between neighboring rows being relatively constant within the array.

**[0060]** It is to be recognized that each device may have one or more arrays, and certain reaction sites may be placed on the same surface as the array but outside the array. For instance, certain reference sites or fiducial features for positioning purpose may be placed outside of the normal array arrangement. In another aspect, the first surface of the planar waveguide may also contain a reference site for calibrating the intensity or uniformity of the light provided into the planar waveguide from the light source. The reference site may contain an excitable molecule immobilized on a portion of the first surface and may be located proximal to the array or being part of the array. In another aspect, the reference site may be formed during execution of an assay. By way of example, a human IgG spot printed on the first surface may serve as the reference site in an assay that uses fluorophore-labeled anti-human IgG as the detection reagent.

**[0061]** In another embodiment of the disclosure, the array on the first surface of the waveguide contains one or more negative control sites, wherein at least one of such negative control site is formed by printing onto the first surface a composition that does not contain any molecule that detectably binds to any analyte in the sample. In one aspect, a composition containing only the buffer or solvent may be printed onto the first surface to form a negative control site. In another aspect, a negative control site may contain a molecule that is known to not interact with the analytes of interest in the sample. For instance, for the detection of antibodies against HIV in a human blood sample, a composition that does not contain any molecules known to interact with the human anti-HIV antibodies directly or indirectly may be printed onto